CLAIMS

WE CLAIM:

1. A method of detecting a donor-product of a group transfer reaction, the method comprising:

a) reacting an activated form of a donor with an acceptor in the presence of a catalytically active enzyme;

b) forming the donor-product and an acceptor-X;

c) contacting the donor-product with a first complex comprising a detectable tag capable of producing an observable;

d) competitively displacing the detectable tag of the first complex by the donor product to generate a second complex and a displaced detectable tag; and

e) detecting a change in the observable produced by the detectable tag in the first complex and the displaced detectable tag.

2. The method of Claim 1, further comprising,

f) quantifying the observable of step (e).

3. The method of Claim 1, wherein,

a) the activated form of the donor comprises donor-X;

b) the acceptor comprises a substrate for the catalytically active enzyme, wherein the substrate is selected from the group consisting of a polypeptide, a protein, a nucleic acid, a lipid, a carbohydrate and a small molecule substrate;

c) the donor-product comprises a nucleotide or a non-nucleotide, wherein the non-nucleotide is a metabolic intermediate selected from the group consisting of s-adenosylhomocysteine, nicotinamide or coenzyme A;

d) the acceptor-X comprises a reaction product in which X is a covalent adduct; wherein the covalent adduct is selected from the group consisting of a phosphate, a sulfate, a carbohydrate, a naturally occurring amino acid, a synthetically derived amino acid, ADP-ribose, a nucleotide, a methyl, an acetyl, and a glutathione moiety; and wherein the covalent adduct is optionally capable of altering either the function, the stability, or both the function and the stability of the acceptor;

e) the first complex comprises a macromolecule and a detectable tag; and

f) the second complex comprises the macromolecule wherein the detectable tag is competitively displaced by the donor-product resulting in the production of an observable.

4. The method of Claim 3, wherein the macromolecule is selected from the group consisting of an antibody, a polypeptide, a protein, a nucleic acid molecule, and an inactivated enzyme that is capable of contacting the donor-product with high affinity.

5. The method of Claim 4, wherein the antibody is a monoclonal antibody, a polyclonal antibody, or a recombinant antibody.

6. The method of Claim 4, wherein the antibody is specific for the donor product, and wherein the level of antibody cross-reacting with the donor-X is less than the level of specificity that the antibody exhibits towards the donor-product.

7. The method of Claim 1, wherein the detectable tag is a tracer, wherein the tracer is a fluorescent or a chemiluminiscent molecule conjugated to a nucleotide or a non-nucleotide.

8. The method of Claim 1, further comprising detecting a catalytic activity, wherein the catalytic activity generates the donor-product in the group transfer reaction.

9. The method of Claim 8, wherein the catalytic activity comprises a chemical catalytic activity, an enzymatic activity, or a combination thereof; wherein the enzymatic activity comprises a sulfotransferase, a kinase, a UDP-glucuronosyltransferase, a methyl transferase, a acetyl transferase, a glutathione transferase, and a ADP-ribosyltransferase.

10. The method of Claim 1, wherein the method is an immunoassay.

11. The method of Claim 10, wherein the immunoassay is selected from the group consisting of fluorescence polarization immunoassay (FPIA), fluorescence resonance energy transfer (FRET), enzyme linked immunosorbant assay (ELISA), chemiluminescence immunoassay.

12. The method of Claim 1, wherein the method is used for screening a chemical library to identify a molecule which is capable of activating or inhibiting a group transfer reaction enzyme.

13. The method of Claim 12, wherein the molecule is capable of altering either the function, the stability, or both the function and the stability of the acceptor.

14. The method of Claim 12, wherein the molecule is capable of exhibiting a therapeutic effect.

15. The method of Claim 12, wherein the library is screened using a high-throughput screening technique comprising a multiwell plate, a microarray or a microfluidic system.

16. An antibody produced against a donor product of a group transfer reaction, wherein the antibody comprises the ability to preferentially distinguish between a donor-product and a donor in the presence of a high donor concentration.

17. The antibody of Claim 16, wherein the donor-product is selected from the group consisting of a nucleotide or a non-nucleotide.

18. The antibody of Claim 16, wherein the antibody is specific for a phosphate portion of a nucleotide, and wherein the antibody has the ability to distinguish between a 5'-phosphate, a 5'-phosphosulfate, a 5'-diphosphate and a 5' –triphosphate.

19. A homogeneous competitive binding assay for a donor product of a group transfer reaction, the assay comprising the steps of:

a) combining the donor-product with a tracer and a macromolecule to provide a mixture, the macromolecule being specific for the donor product, the tracer comprising the donor-product conjugated to a fluorophore, the tracer being able to bind to the macromolecule to produce a detectable change in fluorescence polarization;

b) measuring the fluorescence polarization of the mixture to obtain a measured fluorescence polarization; and

c) comparing the measured fluorescence polarization with a characterized fluorescence polarization value, the characterized fluorescence polarization value corresponding to a known donor-product concentration.

20. The assay of Claim 19, wherein the group transfer reaction is catalyzed an enzyme.

21. The assay of Claim 19, wherein the enzyme is selected from the group consisting of a kinase, a sulfotransferase, a methyltransferase a UDP-glucuronosyltransferase, a acetyl transferase, a glutathione transferase, and a ADP-ribosyltransferase.

22. The assay of Claim 19, wherein the donor-product is selected from the group consisting of phosphoadenosine-phosphosulfate (PAP), adenosine diphosphate (ADP), uridine diphosphate (UDP), s-adenosylhomocysteine, nicotinamide, and Coenzyme A.

23. The assay of Claim 19, wherein the fluorophore is selected from the group fluorescein, rhodamine, Texas red and derivatives thereof.

24. A method of using the assay of Claim 19 to screen a chemical library to identify a molecule which is capable of inhibiting or activating a group transfer reaction enzyme.

25. An assay kit for characterizing a donor-product from a group transfer reaction, the assay kit comprising:

a macromolecule and a tracer, each in an amount suitable for at least one homogeneous fluorescence polarization assay for donor-product, wherein the macromolecule is a an antibody or an inactivated enzyme; and wherein the macromolecule and the tracer may be separate or together in the container.

26. The assay kit of Claim 25, further comprising packaging, and instructions for using the antibody and the tracer in the homogeneous fluorescence polarization assay, the antibody being specific for donor-product, the tracer comprising donor-product conjugated to a fluorophore, the tracer being able to bind to the antibody to produce a detectable change in fluorescence polarization.

27. The assay kit of Claim 26 wherein the fluorophore is selected from the group consisting of fluorescein, rhodamine, Texas red and derivatives thereof.